Available online at www.sciencedirect.com
SCIENCE DIRECT®
Insect Biochemistry and Molecular Biology 35 (2005) 837–846
Insect Biochemistry and Molecular Biology
www.elsevier.com/locate/ibmb

Identification of cytochrome P450 and glutathione-*S*-transferase genes preferentially expressed in chemosensory organs of the swallowtail butterfly, *Papilio xuthus* L.

Hajime Ono[1], Katsuhisa Ozaki, Hiroshi Yoshikawa*

JT Biohistory Research Hall, 1-1, Murasaki-cho, Takatsuki, Osaka 569-1126, Japan

Received 3 October 2004; received in revised form 5 March 2005; accepted 18 March 2005

Abstract

The swallowtail butterfly, *Papilio xuthus* L., feeds exclusively on members of the plant family, Rutaceae. Female butterflies lay eggs in response to specific chemicals contained in their host plants. They perceive a variety of polar compounds as oviposition stimulants through the tarsal chemosensilla of the foreleg by drumming upon the leaf surface. We undertook an expressed sequence tag (EST) analysis to identify the chemosensory-related genes that are expressed in chemosensilla on the tarsus of *P. xuthus*. Several genes that showed similarity with biotransformation enzymes were identified from the ESTs. Among them, a cytochrome P450 and a glutathione-*S*-transferase (GST) were preferentially expressed in the chemosensory organs. We have determined the structure of both cDNA and genomic sequences encoding these enzymes and designated the P450 as CYP341A2, a novel member of CYP341A subfamily, and the GST as GST-pxcs1, respectively. We observed a localized expression of CYP341A2 at the base of tarsal chemosensilla by in situ hybridization. These results suggest that these degrading enzymes play a role in the chemosensory reception for host plant recognition.

Keywords: *Papilio xuthus* L.; Cytochrome P450; Glutathione-*S*-transferase; Chemoreception; Tarsal chemosensillum

1. Introduction

Insects perceive a number of chemicals such as pheromones and other semiochemicals for mating, feeding and avoidance of predators. For phytophagous insects, detection of a specific repertoire of plant secondary metabolites by olfaction or contact chemoreception plays an important role in determining whether or not a plant is suitable. In most lepidopteran species, the recognition of volatile and soluble chemicals effects their oviposition behavior including location, orientation, landing and final assessment (Renwick and Chew, 1994). Swallowtail butterflies that belong to the family of Papilionidae selectively utilize a limited number of plants belonging to a single or a few families. They lay eggs on their host plant by detecting specific chemicals referred as oviposition stimulants through tarsal chemoreceptors on their forelegs by drumming on the leaf surface (Feeny et al., 1983; Nishida, 1995). The tarsus of *Papilio xuthus* L. bears sensilla on the ventral side, which are more numerous in the female than in the male. Of more than 100 sensilla present in the female, about 70 are found in the fifth tarsomere. The tarsal sensillum of the butterfly, *Pieris brassicae*, possesses a terminal pore and contains four chemoreceptor cells and one mechanoreceptor cell (Ma and Schoonhoven, 1973) corresponding to the property of *Drosophila* taste sensilla (Stocker, 1994).

*Corresponding author. Tel.: +81 726 81 9752; fax: +81 726 81 9756.
E-mail address: yoshikawa@brh.co.jp (H. Yoshikawa).
[1]Present address: Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455, USA.

0965-1748/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibmb.2005.03.013

The oviposition stimulants have been extensively identified for swallowtail butterfly species in the genus *Papilio*, most of which feed exclusively on the plant families, Rutaceae and Apiaceae. These stimulants encompass a variety of polar compounds that include flavonoids, hydroxycinnamic acids, cyclitols, amino acid derivatives and simple organic bases (Nishida, 1995). Although 10 oviposition stimulants have been identified in *P. xuthus*, (Nishida et al., 1987; Ohsugi et al., 1991), there is no previous studies about the chemoreception of oviposition stimulants at the molecular level.

Biochemical and molecular biological analysis have shown that three types of proteins, pheromone receptors, pheromone binding proteins and pheromone degrading enzymes, are mainly involved in the peripheral reception events in the pheromone reception (Vogt and Riddiford, 1981; Breer, 1996). The process of enzymatic degradation of ligands as well as their reception is necessary in chemoreception to terminate continuous stimulations to receptors. Two types of pheromone degrading enzymes, esterases and aldehyde oxidases, have been characterized in three lepidopteran species, *Antheraea polyphemus*, *Manduca sexta* and *Bombyx mori*, by biochemical analysis. (Vogt and Riddiford, 1981; Vogt et al., 1985; Rybczynski et al., 1989, 1990). Recently, cDNAs encoding putative odorant-degrading esterases were cloned from two lepidopteran species, *A. polyphemus* and *Mamestra brassicae* (Ishida and Leal, 2002; Maïbèche-Coisne et al., 2004a). In the last few years, some biotransformation enzymes that are related to the metabolism of xenobiotics such as pesticides and toxic plant secondary metabolites have been reported as odorant degrading enzymes. A cDNA encoding an olfactory-specific glutathione-*S*-transferase (GST) was cloned from *M. sexta* (Rogers et al., 1999). In *Drosophila*, Wang et al., 1999 found that an UDP-glycosyltransferase, a short chain dehydrogenase/reductase and a cytochrome P450 (P450) were expressed specifically in antenna. Recently, cDNAs encoding two P450s, one of which was specifically expressed in antenna, were cloned from *M. brassicae* (Maïbèche-Coisne et al., 2002). More recently, a P450 specific to male antennae of a scarab beetle, *Phyllopertha diversa*, has been characterized as a pheromone-degrading enzyme by electrophysiological studies (Maïbèche-Coisne et al., 2004b).

We undertook an expressed sequence tag (EST) analysis to identify the chemosensory-related genes expressed in chemosensilla on the tarsus of the swallowtail butterfly, *P. xuthus*. Several biotransformation enzymes, three P450s and five GSTs, were identified from ESTs. Among them, one each of P450 (CYP341A2) and GST (GST-pxcs1) was preferentially expressed in the chemosensory organs such as tarsi, antennae and labella. Moreover, in situ hybridization analysis showed that the expression of the CYP341A2 was localized in the tarsal chemosensory organ. These results suggest that these degrading enzymes have functional roles either in protecting organs exposed to the external environment from toxic plant chemicals or in terminating signals through degradation of ligands such as oviposition stimulants in the tarsal chemosensilla.

2. Materials and methods

2.1. Materials

Eggs of *P. xuthus* were obtained from adults collected from the suburb of Kyoto, Japan. The larvae were raised on leaves of various *Citrus* plants or semi-artificial medium containing *Citrus* leaf powder. The insects were kept at $24 \pm 2\,^{\circ}C$ under a photoperiod of 16L/8D for rearing. Tissues dissected from pupae just before and after eclosion were frozen in liquid nitrogen until use.

2.2. Construction of tarsal cDNA library

Tarsi were manually dissected from 20 pairs of forelegs of female butterflies and then homogenized in liquid nitrogen. Total RNA was extracted from the homogenate using MagExtractor–RNA- (TOYOBO, Tsuruga, Japan) and then treated with DNase I to remove genomic DNA. After DNase I was inactivated, the purified total RNA was converted into double-stranded cDNA using SMART PCR cDNA Synthesis Kit (Clontech, CA, USA). Briefly, first strand cDNA was synthesized with SuperscriptII Reverse Transcriptase (Invitrogen, CA, USA) using an anchored oligo(dT) with adaptor sequence, and then a primer with the same adaptor sequence was annealed to the 5′ cap of the first strand cDNA to synthesize second strand cDNA. The resulting cDNA was amplified by PCR using the primer designed from the adaptor sequence. The amplified cDNAs were size-selected by agarose gel electrophoresis and the resulting fragments ranges from 1 to 5 kb were ligated into the pGEM-T vector (Promega, WI, USA). The ligated mixture was transformed into *E. coli* DH5α cells, and the resulting tarsal cDNA library consisted of 2×10^6 individual clones.

2.3. EST analysis

Clones were picked from the cDNA library at random and the plasmid DNAs were miniprepped using MagExtractor–Plasmid- (TOYOBO). The inserts were sequenced using the primers, (5′-AACAACGCAGAGTACGCGGG-3′) and (5′-TTTTTTTTTTTTTTTTV-3′), that were designed from the adaptor sequence and the oligo(dT) primer used in cDNA synthesis,